interaction of NPT2a with PDZ1 or PDZ2 in full-length NHERF1 was 1.6μM and 33μM, respectively. Only isolated PDZ1 interacted with NPT2a (Kd=11μM). Isolated PDZ1 and PDZ2 interact with PTHR with Kd's of 2.9μM and 1.3μM, respectively. In the context of full-length NHERF1 PTHR binds PDZ1 and PDZ2 with a Kd of 1.7μM and 2.2μM, respectively. We conclude that variances are evident between measurements of isolated PDZ domains and full-length NHERF1. The lower affinity of the PDZ2 domain in full-length NHERF1 compared to isolated PDZ2 is due to adoption of the self-associated conformation. Graded occupancy of PDZ domains may impart differences in NHERF1 signaling and trafficking. We suggest that energetic barriers separating NHERF1 self-associated and open states in the cellular environment may be reduced by phosphorylation. MD simulations predict that upstream residues in the C-terminus contribute to the binding and specificity of interaction with PDZ domains. The results provide novel molecular insights into the recognition of major PDZ binding partners.

3349-Pos Board B77
Molecular Basis of Phosphatidylinositol 4,5-Bisphosphate Recognition by the Adaptor Protein Tirap
Xiaolin Zhao, Shuyan Xiao, Daniel G.S. Capelluto.
Protein Signaling Domains, Department of Biological Sciences, Virginia Tech, blacksburg, VA, USA.
Toll-like receptors (TLRs) provide a mechanism for host defense by activating innate immune responses. Activated TLRs [*e.g.*, by bacterial lipopolysaccharide (LPS)] dimerize, and interact with adaptor proteins through their cytosolic TIR domains to trigger a signaling cascade that ultimately leads to the expression of proteins involved in pro-inflammatory responses. One such adaptor protein is the TIR domain-containing adaptor protein (TIRAP), which contains an N-terminal phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$)-binding region that is required for plasma membrane targeting and a C-terminal TIR domain, which mediates myeloid differentiation primary response gene 88 (MyD88) association. Upon ligand binding, the LPS-binding protein TLR4 is proposed to be recruited to $PtdIns(4,5)P_2$-rich regions where TIRAP resides. At these sites, TIRAP recruits MyD88 to the plasma membrane via TIR-TIR domain interactions; thus TIRAP bridges MyD88 binding to activated TLR4. A conserved short stretch at the N-terminus of TIRAP has been shown to be sufficient to target the protein to the plasma membrane. We show that this region, which we named the $PtdIns(4,5)P_2$ binding motif (PBM), folds in dodecylphosphocholine (DPC) micelles, and binds $PtdIns(4,5)P_2$. The solution structure of the DPC-associated TIRAP PBM indicates that the peptide adopts a helical structure in micelles. Furthermore, we demonstrate that $PtdIns(4,5)P_2$ can induce a helical structure in TIRAP PBM, a feature observed in other $PtdIns(4,5)P_2$ modules. NMR data indicates that TIRAP PBM binds PIP2 in a fast exchange regime with a moderate affinity through two conserved basic regions. Thus, these studies will provide a basis for understanding the mechanism of TIRAP's membrane targeting and recruitment of TIR-containing proteins required to trigger pro-inflammatory signaling.

3350-Pos Board B78
Probing the Origin of Structural Stability of Single and Double Stapled P53 Peptide Analogs Bound to MDM2
Udayan Mohanty.
Department of Chemistry, Boston College, Chestnut Hill, MA, USA.
The alpha-helical conformation and structural stability of single and double stapled all-hydrocarbon cross-linked p53 peptides in solution and when bound to MDM2 are investigated. We determined the effects of the peptide sequence, the stereochemistry of the cross-linker, the conformation of the double bond in the alkene bridge, and the length of the bridge, and the relative stability of the alpha-helix structure. The conformation population distribution indicates a fully helical state and several partially folded states. The distribution of dihedral pairs of the stapled peptides in the bound state indicates a significant population around the alpha-helical region. Peptide residues over which the linker spans tend to have the highest helical occupancy. WaterMap simulations yielded over hundred hydration sites identifying regions with water density greater than twice that of the bulk. The free energy released by displacing the hydration sites populating the staple-binding pocket was determined. The three hydrophobic pockets, which bind Phe19, Trp23 and Leu26, provide a large positive contribution to the binding free energy for all peptides studied. In agreement with experimental data, potential of mean forces and weighted histogram analysis methods indicated the order of peptides from lowest to highest binding affinity to be *cis*-sah3, WT, *cis*-sah8 and *cis*-sah4.
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3351-Pos Board B79
Dynamics of Multifunctional Dehaloperoxidase Hemoglobin
Stefan Franzen, Jing Zhao, Hanna Gracz.
North Carolina State University, Raleigh, NC, USA.
Multi-functional dehaloperoxidase-hemoglobin (DHP) is a versatile protein that functions as an oxygen transporter, but also has functions related to detoxification of a range of substrates including brominated phenols and indoles. It is ironic that the inhibitor binding site has been known for 15 years, but the substrate binding sites remain elusive. Two recent X-ray crystallographic studies reveal internal substrate binding sites. However, since there are at least three binding sites for 2,4,6-trihalophenols alone, the functional location of substrate binding remains unresolved. Moreover, recent kinetic data show that a number of indoles are excellent substrates for DHP and the isotope data indicate that some of these are oxidized directly by bound H2O2 (peroxygenase function) or O2 (monooxygenase function). Clearly the internal binding requires a large binding cavity and protein dynamics that permit the entrance of these large molecules into the distal pocket. Time-resolved X-ray crystallography reveals ballistic motion of carbon monoxide to the only internal Xe-binding site and rapid escape from the protein, both features that are consistent with the open architecture of the distal pocket. Preliminary NMR dynamic studies show that this method can be used to complement X-ray crystallography to provide information on the location of substrate binding. Resonance Raman studies complement these methods as well providing a means to study how ligand binding to the heme Fe interacts with the internally bound inhibitors and substrates. This combination of methods has the potential to reveal functional differences in the binding of different substrates and the role played by protein dynamics in controlling the functional switching of DHP.

3352-Pos Board B80
In Search of Ras Inhibitors
Alemayehu A. Gorfe.
University of Texas Medical School at Houston, Houston, TX, USA.
Membrane binding is essential for the biological activity of Ras proteins, the key regulators of diverse signaling pathways whose malfunction accounts for more than 15% of all human tumors and up to 90% of cases in specific tumor types. However, decades of effort has to date failed to yield selective Ras inhibitors. We hypothesize that disrupting the dynamic coupling between the nucleotide-binding and membrane-interacting regions of Ras by small molecule ligands may have better therapeutic potential. As part of an effort to test this hypothesis, we applied a variety of existing and new ligand binding site identification schemes on ensembles of Ras conformers generated by extensive molecular dynamics simulations in solution and membrane environments. We will discuss the discovery of new pockets that have the potential to bind small molecule ligands, along with alternative strategies for Ras drug design emerging from interactions between various computational and experimental approaches.

3353-Pos Board B81
Ligand Binding Site Identification in Membrane-Bound Oncogenic K-Ras
Priyanka Prakash, Alemayehu A. Gorfe.
Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX, USA.
Ras proteins regulate diverse signaling pathways by cycling between active and inactive conformational states. Ras mutations are associated with a variety of cancers and developmental disorders, accounting for ~15% of all human tumors and 90% of cases in pancreatic cancer. Membrane binding through the hypervariable region is essential for the biological activity of Ras proteins. Understanding isoform-specific differences in membrane binding could therefore lead to new therapeutic strategies. The goal of this work was to find novel druggable sites on the surface of membrane-bound oncogenic K-ras. To this end, we carried out multiple, microsecond-long all-atom molecular dynamics simulations on G12D K-ras and generated a very large structural ensemble. Applying a variety of ligand binding site identification techniques on this ensemble, we identified several pockets that have the potential to bind small molecule ligands. Moreover, we found that some of the previously described ligand-binding sites in solution are inaccessible to ligands in the presence of a membrane. Our analysis also yielded some new insights into the dynamics of membrane-bound oncogenic K-ras.

3354-Pos Board B82
The Prominence of the Ligand Peptide Carboxyl Terminus in the MHC Class I Molecules Stability and Affinity
Esam T. Abualrous[1,2], Sunil Kumar Saini[1], Venkat Raman Ramnarayan[1], Martin Zacharias[3], Sebastian Springer[1].
[1]Jacobs University Bremen, Bremen, Germany, [2]Ain Shams University, Cairo, Egypt, [3]Technical University Munich, Munich, Germany.
Major histocompatibility class I complex (MHC) proteins are intracellular receptors that bind peptides of eight to ten amino acids to present them to the